Chromosome stability of callus cultures of *Crocus sativus*

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ABSTRACT

Two year-old callus cultures, raised from corm explants of *Crocus sativus*, showed an unusual chromosome number stability when maintained on MS medium supplemented with 2,4-D (2 mg/l) and kinetin (0.5 mg/l). Such studies may give an insight into the possible origin of somaclonal variants.

Key words : *Crocus sativus*, callus cultures, chromosome stability.

Crocus sativus, the saffron plant of commerce, is propagated vegetatively through corms. Because it is triploid, inducing genetic variability by conventional breeding is difficult. One of the potential ways to induce genetic variation would be through plant tissue cultures. To induce variation, we developed callus cultures from corms of *C. sativus*. Although plant regeneration from callus raised from corms was reported by Ding *et al.* (1981), Ilahi, Jabeen & Firdous (1987) and Isa & Ogasawara (1988), there is no information available on the genetic stability of the callus. In this communication, we report the chromosome stability of two year old callus cultures of *C. sativus*. Such studies on chromosomal behaviour of callus cultures are important to understand the origin of possible somaclonal variants.

C. sativus corms were obtained from the fields of Kashmir, India. The corms were washed thoroughly after removing the outer layer and sterilized by 0.15% mercuric chloride for 10 min and washed thoroughly with sterile distilled water. Each corm was cut into four to eight pieces depending on the size of the corm and planted aseptically on Murashige and Skoog's (1962) medium supplemented with 3% sucrose, 2,4-dichlorophenoxyacetic acid (2 mg/l) and kinetin (0.5 mg/l). The pH of the me-

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dium was adjusted to 5.9 before autoklaving and sterilisation at 120°C for 20 min. The cultures were kept under continuous illumination. For determination of chromosome number, the calli, a week after subculturing were pretreated with 0.002M 8-hydroxyquinoline at 4°C for 3-4 h. They were washed in water and fixed in 1:3 glacial acetic acid and absolute alcohol. The fixed material was treated with a mordant of 4% iron alum in 45% propionic acid for 15-30 min. Subsequently, the stained material was squashed in 0.5% haematoxylin in 45% propionic acid.

Callus was initiated from corms after about 60 days of inoculation. The callus thus obtained was repeatedly subcultured at 30-40 day intervals on to the same medium. In the present study, two year-old callus was studied for chromosome number stability, because in some, stability decreases with time (Bayliss 1980). Chromosome number of hundred well spread metaphase plates from pretreated cells was determined. Interestingly, all cells were observed to have the chromosome number of 2n=24 (Fig. 1). Such retention of somatic chromosome number was also reported in other genera. More recently, Griesbach (1990) reported an unexpected genetic stability in *in vitro* cultures of day-lily. Although variability in *Crocus* is reported to occur under *in vivo* conditions in rare cases, variation in chromosome number was not observed by these workers (Estilai 1978; Dhar, Sapru & Rekha 1988). Thus, maintaining callus cultures on medium containing 2,4-D, which is otherwise potent in inducing chromosome number variability, did not affect the chromosome number of *C. sativus* callus cultures.

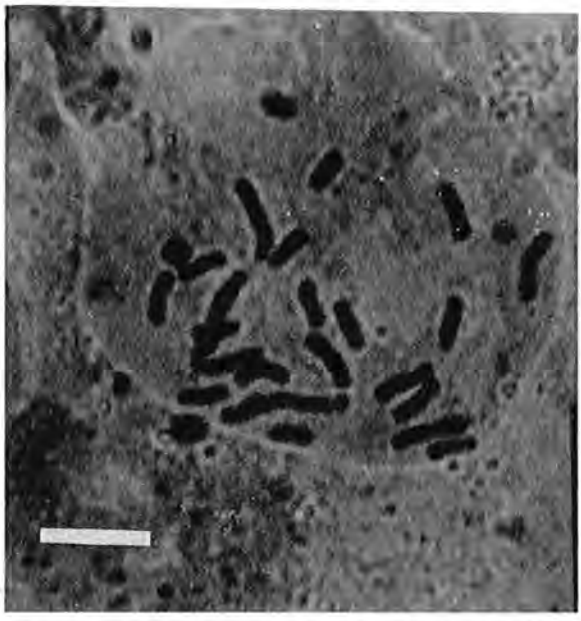

Fig. 1. Metaphase chromosomes of two year-old callus cells of *Crocus sativus* showing the parental chromosome nuber 2n=24 (Bar on the figure represents 10 μm)

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